CC/2020/02

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Second draft revised Guidance Statement (G05): Defining a Point of Departure and Potency Estimates in Carcinogenic Dose Response

1. The COC has periodically published guidelines for the evaluation of chemicals for carcinogenicity. The first guidance was published in 1982 and has undergone several updates since then to reflect advances in development and validation of methods for assessing risk of chemical carcinogenicity.

2. These updates included the separation of the overall guidance into individual documents during 2012 – 2014 to allow faster revisions to be made in the case of rapidly developing areas. This included a separate document addressing points of departure and potency estimates in carcinogenic dose response (G05).

3. Since publication of the first version of G05, EFSA and WHO have jointly reviewed the use of the threshold of toxicological concern (TTC) approach (EFSA and WHO, 2016) whilst EFSA has published new guidance on bench-mark dose (BMD) modelling (EFSA, 2017) and updated guidance on the use of the TTC approach (EFSA, 2019).

4. A revised version of G05 containing these amendments was presented to COC in November 2019 (CC/2019/14) Following discussion it was agreed that the revised version should be further streamlined to produce a standalone document that could be read in isolation but which provided links to other COC Guidance Statements relating to further aspects of the risk assessment process.

5. Since the discussion at the November 2019 meeting, the WHO has published an updated draft of EHC240: Principles and methods for the risk assessment of chemicals in food, for public comment which includes Chapter 5: Dose-Response assessment and derivation of health-based guidance values (WHO, 2019). While this document is a consultation draft, the guidance statement has been revised in line with this in expectation that the draft is unlikely to be significantly changed before being finalised. The Secretariat will keep this under review.

Questions for the Committee

6. Members are asked to:

   i. Comment on the structure of the second draft revised document.

   ii. Comment on whether the revised level of detail included is appropriate.

iii. Highlight any new methods for determining POD that are not currently included. Comment on whether the Committee recommendations are still appropriate.

NCET at WRc/IEH-C under contract supporting the PHE COC Secretariat
March 2020

Committee on CARCINOGENICITY

Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC)

COC Guidance Statement G05 – Second Draft v2-0

Defining a Point of Departure and Potency Estimates in Carcinogenic Dose Response

https://www.gov.uk/government/groups/committee-on-carcinogenicity-of-chemicals-in-food-consumer-products-and-the-environment-coc

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COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Defining a Point of Departure and Potency Estimates in Carcinogenic Dose Response

Contents

1.0 Introduction

1. This guidance statement (G05) forms part of a series by the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC), and should be read in conjunction with these. The Guidance Statement

series aims to provide users with an accessible overview of the various stages of the risk assessment process for chemical carcinogenicity including for regulatory submissions, as advised by the COC.

2. The overall strategy of risk assessment of chemical carcinogenicity is detailed in guidance statement G01 and is based on a four-stage approach, as shown in Figure 1. The term 'hazard' describes the intrinsic capacity of a chemical to cause an adverse effect on human health, such as cancer. 'Risk' is the probability that the adverse health effect will occur. When a carcinogenic hazard is identified, the level of risk will depend on circumstances such as the nature and degree of exposure to the chemical in question.

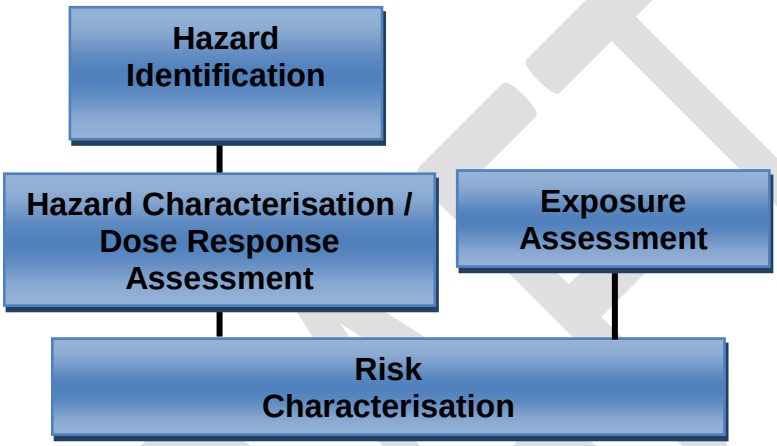

Figure 1: Four stage approach to the risk assessment, after the US National Academy of Sciences, 2005.

3. Hazard identification involves a qualitative description of the nature of the hazard, and hazard characterisation provides a quantitative description of the change in effect caused by differing doses of a chemical substance after a certain exposure time, i.e. the dose-response relationship. The purpose of analysing the dose-response relationship is to estimate the response and, ultimately, the risk from the levels of exposure to the chemical in the environment, food etc.

4. The relationship between dose and response may be used to aid hazard characterisation by allowing a comparison of carcinogenic potency. These estimates give an indication of the dose of a substance (administered over a standard animal lifespan) that results in a fixed incidence (e.g. 5, 25 or 50%) of tumours, after correction for the spontaneous background incidence of tumours among controls (Barlow et al., 2006). The possible impact of human-specific factors on the dose-response relationship established in experimental species, should always be considered; these include species differences in absorption, distribution, metabolism and excretion (ADME), mode of action and variability in susceptibility between species (inter-species) and within humans (intra-individual).

5. There are a number of methods for the characterisation of hazard based on whether a carcinogen acts via a genotoxic or non-genotoxic mechanism. However, both types of carcinogen can be classified with regard to tumourigenicity on the basis of potency. Although potency is ideally represented by the overall position and shape of the dose-effect or dose-response curve, the value (dose) at a particular point on the curve is most often used as a surrogate. This point, also known as the point of departure (POD) or reference point is the starting point for risk characterisation, whether using a margin of exposure approach or deriving a health-based guidance value (see G06 for more information).

6. An example POD is the dose level associated with a tumour incidence that is 10% above that in the control group. The Committee recognises that where data on tumourigenicity *per se* are lacking, it may be possible to use continuous data such as specific DNA damage observed in target organs as a surrogate measure of response, for determining a POD.

7. This Guidance Statement (G05) provides an overview of the various methods for deriving PODs and potency estimates associated with exposures to chemical carcinogens. The tools outlined are those that are available to use *when considered appropriate by the risk assessor*. The derivation and use of POD including the No Observed Adverse Effect Level / Lowest Observed Adverse Effect Level (NOAEL/LOAEL; section 2.1) Benchmark Dose (BMD; section 2.2) and potency estimates such as the TD50 (section 2.4) and T25 (section 2.5), to estimate the relative potency of carcinogens is described. This guidance document also details the Threshold of Toxicological Concern (TTC) approach which is a 'pragmatic screening and prioritisation tool' that can help the assessment of chemicals for which there is a known structure but a lack of chemical-specific toxicity data, and for which exposure can be estimated.

8. It should be noted that there is no difference in the methodology used for determining PODs for genotoxic and non-genotoxic carcinogens. It is how the dose-response relationship and the POD are used in the final assessment of risk (risk characterisation in Figure 1 above) that varies, depending on whether or not a carcinogenic response occurs through a genotoxic (threshold and non-threshold) or non-genotoxic (threshold) mode of action (see Guidance Document G06 for further discussion of Cancer Risk Characterisation).

2.0 Points of Departure and Potency Estimates

2.1 The NOAEL (No Observed Adverse Effect Level) approach

9. For the majority of toxicological effects, with the exception of most genotoxic effects or where extensive testing has failed to identify a threshold (e.g. in the case neurotoxicity for lead), it is generally assumed that there is an exposure threshold below which no adverse effects occur. The highest administered dose at which no statistically significant adverse difference from the concurrent control group is observed is designated the No Observed Adverse Effect Level (NOAEL) and is often

used as a POD in risk assessments. Use of a NOAEL, instead of a No Observed Effect Level (NOEL) in risk estimates ensures that the assessment is based on adverse effects rather than on minor or adaptive effects.

10. If a statistically significant adverse effect compared to the control group is observed at all dose levels however, the lowest dose used in the study, i.e. the LOAEL (Lowest Observed Adverse Effect Level), may be used as the POD.

11. Typically, the NOAEL (or if one is not available, the LOAEL) is determined for the most sensitive, human relevant effect identified in epidemiological studies or from sub-chronic or chronic studies in experimental species.

12. Although the NOAEL has been widely used as a POD for many years by risk assessors, a number of limitations have been identified (WHO, 2019). One major limitation is considered to be the constraint that the NOAEL has to be one of the applied experimental doses. As a result, dose spacing, the shape of the dose-response curve, the number of animals per group, or the statistical variation in the response and its measurement, are not considered. A consequence of this is that studies with low power (e.g. small group sizes) and/or insensitive methods may only detect relatively large effects resulting in higher NOAELs than a better designed study with appropriate power and/or sensitivity to detect effects (IPCS, 2009a). This may then impact on the risk characterisation process.

2.2 Benchmark Dose (BMD) approach

13. The BMD_x is defined as the dose that corresponds to a specific change (x%) in response compared to the (modelled) response in control animals, the benchmark response (BMR) (Crump, 1995). The BMD is determined by fitting a range of “best fit” mathematical curves to the dose-response data over the range of observable responses from animal studies or human studies (if available), using a selection of different models. From each statistically acceptable modelled dose–response curve, values for the BMD and the lower and upper bound 95% confidence limits (BMDL and BMDU) are obtained. To take experimental uncertainty into account, the lower 95% confidence bound on the benchmark dose ($BMDL_x$) is used as the POD. Figure 1 illustrates the BMD approach (EFSA, 2017).

14. EFSA note that ‘the BMR is not defined as a change with regard to the observed mean background response, but with regard to the background response predicted by the fitted model’. This means that generally the fitted curve does not match the observed background response exactly. There are a number of steps involved in applying the BMD approach which include:

a. Specification of an endpoint(s) and selection of the data type, e.g. individual data points (preferred) or summary data (mean; SD; sample size).

b. Specification of a BMR - predetermined level of change in response relative to controls; typically set at the lower end of the range of responses

that can be detected experimentally, or the observations in epidemiological studies.

c. Fitting of a set of dose-response model(s) and calculation of the BMD confidence interval (BMDL – 95% confidence interval) for each to give a set of BMDLs. Selection of models is dependent on the endpoint (quantal or continuous) and the experimental design used to generate the data (number of dose groups and, for example nested study design).

d. Derive a single BMDL from the set available, preferably by model averaging (see paragraph 17). Where a range of endpoints have been identified an overall study BMDL is selected based on the most critical endpoint.

15. Model selection and model constraints are important considerations in BMD estimation and should be clearly recorded and justified. For model selection, an important criterion is that the selected model should adequately describe the data, especially in the region of the BMR.

16. Once the selected models have been fitted to the data, a series of scientific judgements must be made to ensure the fitted models adequately describe the data. Different types of statistical testing can be utilised to assess the adequacy of model fit. The EFSA guidance recommends that the Akaike information criterion (AIC) should be used to characterise goodness of fit (EFSA, 2017). This is a measure of the degree of fit weighted by the number of free parameters in the model. For dichotomous data, the US EPA software employs Pearson's chi-squared goodness of fit test (US EPA, 1995).

17. It is often the case that a number of models will adequately fit the data, as judged on statistical considerations. In such cases EFSA recommends Model Averaging (Wheeler and Bailer, 2007) as the preferred approach, combining results from each of the fitted models to establish a final BMD confidence interval. However, selection/rejection of models based on AIC value or lowest BMDL can be considered as a sub-optimal alternative in situations where Model Averaging tools are not available (EFSA, 2017).

18. Although the current international guidelines for experimental study design (e.g. OECD TGs) have been developed with the NOAEL approach in mind, they offer no obstacle to the application of the BMD approach. The current guidelines may, however, not be optimal given that the BMD approach allows for more freedom in balancing between number of dose groups and group sizes (Slob, 2014). As these guidelines are revised, e.g. within the OECD Test Guidelines Programme, the possibility to recommend study designs in future that tend to result in better dose–response information (e.g. more dose levels with the same total number of animals) should be taken into account.

2.3 Comparing NOAEL and BMD methodologies for use in risk assessment

19. The BMD approach has a number of advantages over the NOAEL approach in that it makes more complete use of the available dose–response data, takes into account the shape of the dose-response curve more explicitly and is less dependent on dose spacing. BMD also enables quantification of the uncertainties in the dose-response data using statistical methodology (EFSA, 2017). EFSA and the WHO recommend the BMD approach for deriving a POD to be used as a starting point for human health risk assessment, for all endpoints, including carcinogenicity by a genotoxic or non-genotoxic mode of action.

20. Despite the adoption of the BMD approach for determining a POD, there continues to be a need for the NOAEL/LOAEL approach. Not all data sets are amenable to BMD modelling, such as those resulting from incomplete data availability or from a lack of models that can describe a dataset adequately (US EPA, 2012) and the NOAEL approach can be used in this instance. A typical situation where the NOAEL approach is applicable, whereas the BMD approach is not, is when there is a response only in the highest dose group.

21. Once the POD is derived, the assessment moves to the risk characterisation stage which brings together hazard identification and hazard characterisation and the exposure assessment process (see Risk Characterisation Guidance Statement G06).

2.4 The TD50 approach

22. The TD50 (Peto et al., 1984) was originally defined as *the chronic dose rate inducing tumours in a given target site(s) in 50% of experimental animals at the end of a standard lifespan for the species, provided there was no tumour incidence in control animals*. However, as the tumour(s) of interest often occur in control animals, the TD50 definition was modified to *the daily dose rate required to halve the probability of remaining without tumours at the end of a standard life span*. TD_{50} values have been estimated for chemicals listed in the Carcinogenic Potency Database (CPDB) developed by Gold and Zeigler (https://toxnet.nlm.nih.gov/cpdb/cpdb.html, accessed 29/09/19) (Gold et al., 1984, 1997).

23. The TD50 concept is based on the assumption that there is linearity between dose and hazard until tumour onset, which may be complicated by premature deaths from causes other than tumour formation. The concept also depends on the assumption that tumour onset times are observable prior to mortality and, as a result, the approach relies heavily on careful observation of the animals. Tumours that are discovered after death within the study period may cause confounding between mortality and tumour onset and would ultimately result in a biased TD50 estimate. Alternatively, tumours that do not significantly alter survival and remain undiscovered until death would result in the TD50 value relating to the ‘rate of death with tumour’, rather than the tumour incidence rate. This undermines the objective of the carcinogenicity study, which is to evaluate tumour incidence. A description of the

TD50 methodology and the complex statistical analysis involved in its derivation is provided at http://toxnet.nlm.nih.gov/cpdb/td50.html (accessed 29/09/19).

24. The Committee reiterates its previous position that the TD50 is a practical quantitative estimate of carcinogenic potency for the ranking of genotoxic carcinogens, but not for deriving a POD.

2.5 The T25 approach

25. The T25 is defined as the dose eliciting a 25% increase in the incidence of a specific tumour at a selected site above the background level within the standard lifespan of that species (Dybing et al., 1997; Sanner et al., 2001). The methodology does not require the application of complex statistical methods and is determined by simple linear interpolation of data and, in some cases extrapolation beyond the experimental dosing points, preferentially from long-term carcinogenicity bioassays. The minimum data requirements to calculate a T25 are one incidence level significantly greater than the controls (Gillespie et al., 2011). The T25 is influenced by the quality of the bioassay information (e.g. design and evaluation of studies) and factors such as time to first tumour, the influence of toxicity on tumour induction and mortality, and the approach taken regarding statistical analysis of tumour data.

26. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has evaluated the use of T25 estimates for regulatory risk assessment of non-threshold carcinogens (ECETOC, 2002). They report that there may be uncertainties regarding the application of the T25 for potency ranking, particularly with regard to selection of the most sensitive site relevant for humans, the relevance of rodent tumours for humans, and different cancer susceptibilities between rodent species (ECETOC, 2002). The T25 is also the method used by the EU to assess relative potency for the setting of specific concentration limits of preparations and mixtures (EC, 1999). Using the T25 method, Sanner and Dybing (2005) reported a good correlation between the values obtained based on human epidemiological data and those based on experimental data, although the data available for comparison was limited. Previously, the T25 approach has been used in risk assessment for regulation of non-food, genotoxic carcinogenic chemicals in the EU (EFSA, 2005).

2.6 Comparing BMD and T25 methodology for use in risk assessment

27. T25 and the BMD methodology differ in that the T25 is calculated from one data point on the dose-response curve whereas the BMD is derived from dose-response modelling of all available data on the dose-response curve (EFSA, 2005).

28. Although primarily used in carcinogenic potency estimates, the T25 approach can also be used to derive a POD. For example, although the European Chemicals Agency (ECHA) prefers $BMDL_x$ as a POD, if the data do not permit BMD analysis, it suggests that the T25 can be used. This may be particularly applicable to older data sets which may be limited in scope.

29. Dybing et al. (2008) compared the Margin of Exposure (MOE), the numerical value obtained by dividing a POD on the dose-response curve by estimated human exposure to the chemical, for 6 substances obtained using either the $BMDL_{10}$ or the T25. They found that MOEs obtained using the T25 as the POD were on average around 2.35 times higher than those derived using the $BMDL_{10}$ as the POD (Dybing et al., 2008). Benford et al. (2010) compared MOEs for 12 substances in food that are genotoxic and carcinogenic (5 of which were the same as those examined by Dybing et al., 2008) and found that the ratio of MOEs derived from a T25 value varied from those using a $BMDL_{10}$ value by between 0.9 and 4.6, with a mean of 2.9 and a median of 2.6. These results were in line with the expected ratio of 2.5 to account for the 25% vs. 10% risk, assuming linearity in the dose-response relationship, when comparing the T25 with the $BMDL_{10}$ (Benford et al., 2010).

30. In the Committee's discussion of the MOE approach for G06, the guidance document on cancer risk characterisation methods, the Committee considered the use of the BMD approach as a means of deriving a POD to be superior to that of the T25.Therefore, in the event that it is not possible to derive a $BMDL_{10}$, the Committee does not recommend the routine use of the T25 for risk characterisation.

3.0 Potency Ranking of Genotoxic Carcinogens

31. Relative potency estimates could have some pragmatic use in carcinogenic risk assessment as an aid in the prioritisation of genotoxic carcinogenic substances but are not considered adequate for quantifying cancer risks. The uncertainties inherent in potency ranking mean that relative potencies should not be over-interpreted. For example, it is unclear whether the relative ranking identified in the observed dose range would be maintained at low doses, and whether the relative potency in animal studies would be applicable to humans.

32. Data from animal bioassays can be used to rank carcinogenic potency without reference to human intake. Carcinogenic potency estimates, as described in paragraph 3, make use of the available dose-response data, and the POD can be derived from TD50, T25 or BMD approaches for use in potency ranking. For example, in a series of publications Gold and colleagues tabulated data on a large number of compounds allowing their carcinogenic potencies to be expressed as the TD50 (Gold et al., 1997). These values can be used to indicate the relative potencies of a series of compounds.

33. Potency Equivalence Factors (PEFs) have been suggested in circumstances where there is a good surrogate compound for comparison, e.g. inhalation of polycyclic aromatic hydrocarbons (PAHs) (Collins, 1998; Pufulete et al., 2004). Pufulete et al. (2004) suggested that an approach based on PEFs could be developed to include highly potent carcinogenic PAHs provided an appropriate reference data set for relevant PAHs using a route acceptable for inhalation risk assessment is selected. The US EPA (2010) also developed an approach to assessing cancer risk for PAH mixtures using relative potency factors (RPFs), which estimates the cancer risk of individual PAHs relative to that of benzo[a]pyrene (BaP).

The US EPA suggests that these RPFs are applicable to all routes of exposure but acknowledges that there is appreciable uncertainty in doing this. The COC notes that PHE has adopted a surrogate marker approach rather than the use of PEFs for assessment of the public health risk of PAHs in contaminated land (PHE, 2017).

34. Comparing the TD50 and T25 approaches for estimating potency, the TD50 has an advantage in that it takes account of effects of chemicals on survival, however it requires specific software to undertake its derivation. In contrast, the T25 is quick and easy to calculate. There is evidence of a good correlation between rank order produced by TD50 and T25 (Dybing, 1997). In 2006, the COC compared the TD50 with the T25 in an attempt to develop an approach for potency ranking of genotoxic carcinogens for single exposure. Very limited data were available for this purpose and little correlation was found among those substances for which it was possible to obtain chronic TD50 and T25 values, compared to acute T25 values (COC, 2006).

35. The Committee acknowledges that the T25 approach can be used in potency ranking of genotoxic carcinogens but is of the view that the statistics should not be over-interpreted. The reason for this is that there are a number of basic uncertainties, such as whether the relative ranking identified in the observed dose range would be maintained at low doses, and whether the relative potency in animal studies would be applicable to humans. Currently, there is no need to use the T25 to rank non-genotoxic carcinogens, for which tolerable exposure levels can be derived using an approach based on knowledge of mode of action, identification of a NOAEL, and the use of uncertainty factors.

4.0 The Threshold of Toxicological Concern (TTC)

36. The TTC approach is used to screen and prioritise the risk assessment of substances with a known chemical structure but no, or little, specific toxicity data. Application of the TTC approach has been most widely applied to the oral route of exposure and, as such, the following sections focus on that route. Application of the TTC approach to inhalation and dermal exposure routes is not as widely applied but has been considered by SCCS/SCHER/SCENIHR (2012). For the TTC approach to be applied, the estimated exposure of humans to the substance via the oral route should be low (EFSA, 2019).

37. When considered by the COC for the 2004 version of their guidelines, the application of the TTC to carcinogens was a relatively new approach and the Committee concluded that:

> "*careful consideration was needed of the biological, analytical and mathematical issues as well as a much wider database for validation. The Committee consider that it should not currently be used as a generic approach, as the proposed exclusions covered some important classes of genotoxic carcinogens (such as aflatoxin-like compounds, azoxy compounds and N-nitroso compounds) and a number of classes of other carcinogens,*

> *such as heavy metals and TCDD (Kroes* et al., *2004). However, as it is based on ranking by theoretical risk and exposure the Committee agree that it could be used, along with hazard identification and characterisation data, for prioritisation of chemicals, particularly for chemicals that are not subject to regulatory approval schemes."* (COC, 2004).

Since 2004, experience with the application of the TTC approach has increased, and the approach itself has been refined, including proposals for use both for inhalation (Carthew et al., 2009; Escher et al., 2010, Tluczkiewicz et al., 2016) and dermal (Safford et al., 2008; Safford et al., 2011; Safford et al., 2015; Roberts et al., 2015) exposure. The TTC approach has been reviewed by EU committees (SCCS/SCHER/SCENIHR, 2012; EFSA, 2012b; EFSA/WHO, 2016; EFSA, 2019). A paper describing the development of the TTC concept since its introduction in 1995 and the respective EU committee opinions was presented at COC in 2012, and a further update was given to the Committee in 2017 (Paper CC/2012/18, available from: http://webarchive.nationalarchives.gov.uk/20140506122048/http://www.iacoc.org.uk/papers/index.htm and CC/2017/21, available from: https://www.gov.uk/government/groups/committee-on-carcinogenicity-of-chemicals-in-food-consumer-products-and-the-environment-coc#meetings).

38. EFSA (2019) has recommended that the TTC values should be expressed per kg body weight so that they are applicable to different age groups, differing in body weight. It is considered that at oral lifetime exposures below the respective TTC value there is a low probability of any risk, even for a chemical with little or no toxicological data.

39. The Cramer decision tree (1978) distinguishes compounds with structures indicating low oral toxicity (Class I) from those where the structure allows no presumption of safety to be made or indicates significant toxicity (Class III). Class II is an intermediate class where compounds do not have the characteristics of Class III, but neither are they innocuous (Cramer et al., 1978).

40. In their analysis, Munro et al. (1996) utilised a dataset comprising repeat dose oral toxicity data for 613 organic chemicals with 2941 associated No Observed Effect Level (NOEL) values derived from a variety of non-cancer endpoints from sub-chronic, chronic, reproductive and developmental toxicity studies carried out in rodents and rabbits. The 5th percentiles of the NOELs, grouped according to their respective Cramer et al. (1978) class (i.e. Class 1, 2 or 3), were used to derive TTC values of 1800 µg/person/day (30 µg/kg bw per day) for Class I chemicals, 540 µg/person/day (9 µg/kg bw per day) for Class II chemicals and 90 µg/person/day (1.5 µg/kg bw per day) for Class III chemicals, respectively.

4.1 Initial considerations prior to applying the TTC decision tree

41. Prior to its use, it is important to confirm that the substance of interest is suitable for application of the TTC approach. Literature searches are required to

evaluate the level of data available (including using read-across) to perform a risk-assessment. If the group of chemicals within which the substance sits has well-established toxicity data, then the TTC approach should not be used. In addition, substances falling under certain regulations are excluded from use of the TTC where they require submission of toxicity data for approval.

42. During development of the TTC approach, a number of substances were excluded. Current exclusion categories are: groups of potent genotoxic carcinogens, aflatoxin-like, azoxy- or *N*-nitroso substances and benzidines, metals in elemental, ionic or organic form, metal-containing compounds, other inorganic compounds, substances known or predicted to bioaccumulate (for example, polyhalogenated-dibenzodioxins, -dibenzofurans and -biphenyls), proteins, substances with a steroid structure, nanomaterials, radioactive substances and organosilicons (EFSA, 2019).

43. The application of the TTC approach to mixtures requires evaluation on a case-by-case basis. Where all components are known, EFSA recommend a tiered approach to risk assessment, with the assumption of dose addition as a starting point. In the case of mixtures that are not dully defined, the TTC approach may be used provided that analysis has shown that excluded compounds are not present. Under these circumstances, the unknown compounds are considered to be potentially DNA reactive and the sum of the mixture components is evaluated against the lowest TTC value (0.0025 µg/kg bw/day). In circumstances where there are no excluded compounds present, there is no concern for unknown components with regards to DNA reactivity and no organophosphates or carbamates present, the mixture is classed as Cramer Class III.

4.2 Estimates of exposure

44. EFSA recommend that chronic exposure is estimated using the upper end of the distribution range from dietary exposure assessments; where this is unavailable, use of the maximum reported level is suggested. Consideration should be given to subgroups of the population whose dietary exposure may be higher (for example infants and children). In cases of acute exposure (i.e. < 24 h) EFSA advices, where data is available, to use the highest percentile levels in conjunction with high percentile food consumption. If data is unavailable then, as previously, the maximum reported level should be used.

4.3 Application of the TTC decision tree

45. Kroes et al. (2004) developed a decision tree for application of the TTC approach to chemicals in food through combined considerations of structural alerts for genotoxicity with the approach developed by Munro et al. (1996) for *de minimis* exposure values for non-cancer endpoints, based on the structural classification scheme of Cramer et al. (1978). This scheme was proposed for use by EFSA in 2012 and a revised scheme recommended by EFSA and WHO (2016). The latest version of the EFSA/WHO decision tree, given as part of the most recent EFSA guidance (EFSA, 2019), is shown for information in Figure 2.

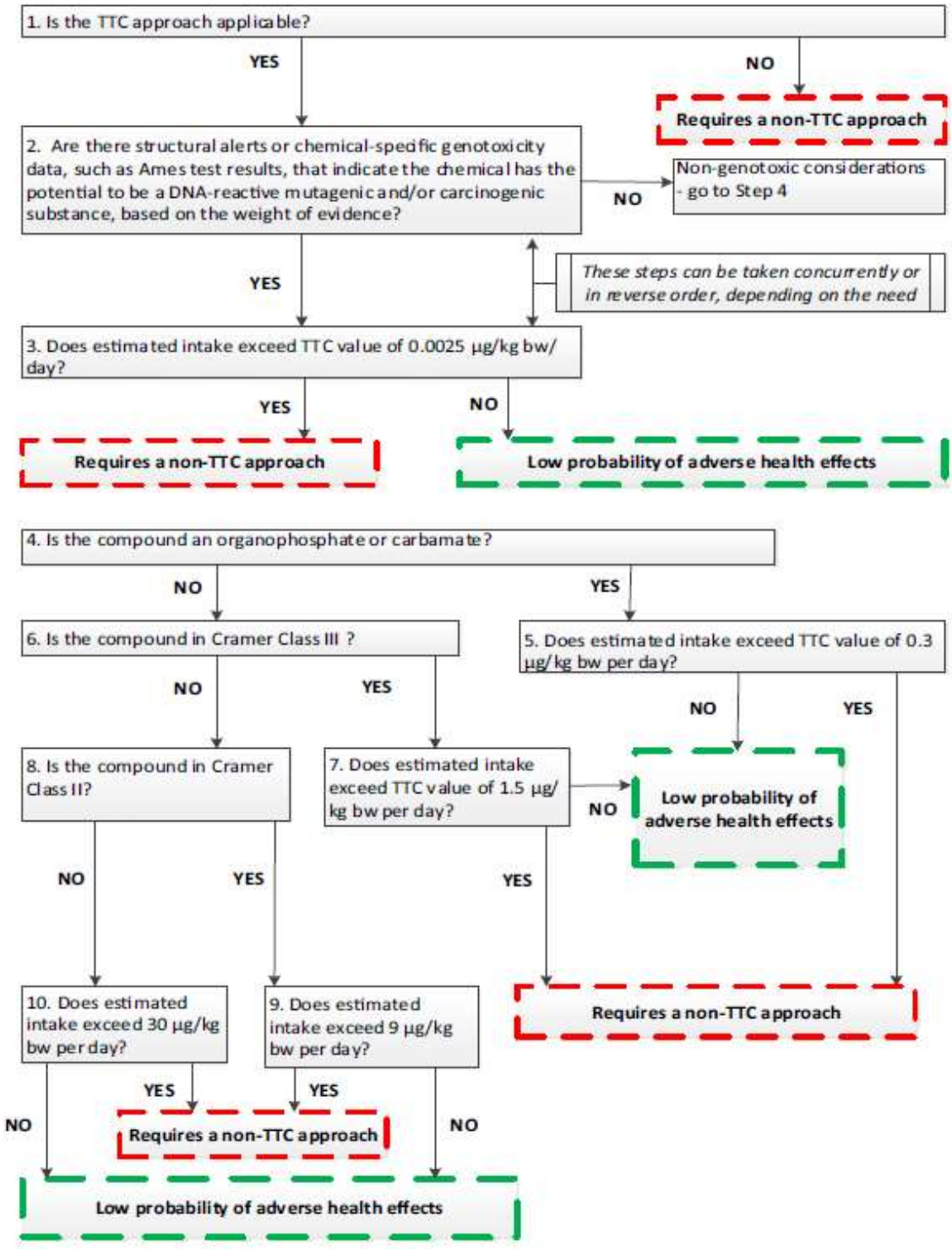

Figure 2: EFSA TTC Decision Tree (EFSA, 2019)

46. Instructions and considerations for carrying out each of the steps in the decision tree are detailed in the EFSA guidance (EFSA, 2019). Of particular relevance to the risk assessment of chemical carcinogens is the assessment of the potential for DNA-reactive mutagenicity or carcinogenicity in Step 2.

47. For all chemicals of interest assessed using the framework, the estimated exposure is compared against an appropriate TTC value. For DNA-reactive mutagens or carcinogens the TTC value is 0.0025 µg/kg bw/day and for organophosphates or carbamates, 0.3 µg/kg bw/day. All other chemicals are grouped according to their Crammer Class, with TTC values of 30, 9, 1.5 µg/kg bw/day for Classes I, II and III respectively.

48. Where the estimated exposure to the chemical of interest is below the appropriate TTC value, it is considered that the probability to cause harm to humans is low. However, if the estimated exposure is higher than the TTC value, it is recommended that a non-TTC approach be adopted to reach a conclusion as to the potential for harm (EFSA, 2019).

4.4 Special considerations in applying the TTC decision tree

49. Exposure estimates in infants under the age of 16 weeks require additional considerations to be applied and these have been discussed fully by EFSA (EFSA, 2017). In addition, differences in dietary exposure and reaction to certain substances in the diet between infants, children and adults are possible and have also been discussed fully by EFSA (EFSA, 2019).

4.5 Regulatory use of the TTC approach

50. In 2009, Felter et al. proposed further refinements to the TTC decision tree, including consideration for chemicals that have structural alerts for genotoxicity but negative data from genotoxicity tests. They proposed using a higher threshold value of 1.5 µg/person/day as an appropriate TTC exposure limit in such cases (Felter et al., 2009).

51. TTC values derived from the Cramer et al. classes are used by EFSA and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for assessing flavouring substances in food (EFSA CEF Panel, 2010). Other uses by EFSA across their remit have included assessments of: impurities, metabolites and degradation products of food additives (EFSA ANS Panel, 2012); pharmacologically active substances present in food of animal origin (EFSA CONTAM Panel, 2018); metabolites and degradation products of plant protection products in the context of residue definition for risk assessment (EFSA PPR Panel, 2016); derivation of ‘maximum acceptable feed concentrations’ for flavouring additives based on default values for feed consumption (EFSA FEEDAP Panel, 2017); development of the criteria for the safety evaluation of mechanical processes to produce recycled poly(ethylene terephthalate) (PET) intended to be used for manufacture of materials and articles in contact with food (EFSA CEF Panel, 2011).

52. The concept of a staged TTC was proposed by Müller et al. (2006) which takes into account the duration of exposure as a key factor impacting on the probability of a carcinogenic response. In 2010, the European Medicines Agency (EMA) agreed to the use of a staged TTC approach during clinical development of medicines for a less than lifetime exposure and recommended limits for daily intake

of genotoxic impurities (GTIs) of 1.5, 5, 10, 20 and 60 µg/day for greater than 12 months, 6-12 months, 3-6 months, 1-3 months and less than 1 month periods, respectively. For single doses, an intake of 120 µg/day was considered to be acceptable from a safety perspective (EMA, 2010).

53. A TTC of 1.5 µg/day is used as part of a staged assessment for the acceptability of known genotoxic impurities present in pharmaceuticals; this is considered appropriate as a risk of 1 in 10^5 (assuming linear extrapolation) is considered acceptable for human medicines (EMA, 2006). The use of a TTC of 1.5 µg/day by the EMA also applies to compounds that show evidence of genotoxicity in *in vitro* tests. A similar approach is used for genotoxic constituents of herbal medicinal products/preparations (EMA, 2008).

54. The TTC approach has also been proposed for use with assessing household and personal care products (Blackburn et al., 2005), skin sensitising substances (Safford, 2008) and for industrial chemicals assessed under REACH (ECHA, 2008).

4.6 TTC endorsement by sister committees

55. The COM published a statement in April 2012 on the genotoxicity testing and hazard assessment of impurities. As part of this, Members agreed that the TTC was a useful concept in identifying impurities requiring genotoxicity assessment, although reference needed to be made to the excluded classes of most concern, e.g. aflatoxin-like, azoxy and N-nitroso compounds, which are potent genotoxic carcinogens (COM, 2012).

56. The COC endorses the views of the COM and the views of EFSA and the SCCS, SCHER and SCENIHR Committees on the TTC.

5.0 Summary

57. The Committee recommends the use of the BMDL as the POD for all carcinogens (see also G03). For genotoxic carcinogens, the likeliest use of the BMDL would be to calculate a MOE as outlined in Guidance Statement G06. For non-genotoxic carcinogens, the BMDL can be used to establish guideline values such as TDI/ADI using uncertainty factors, if carcinogenicity is the critical endpoint. If a BMDL cannot be set for a chemical, the Committee agrees that, although it might be possible to derive a T25 from the dataset, this is not recommended. Instead a NOAEL can be adopted for non-genotoxic compounds, and even for genotoxic compounds noting that this should be used in a way that doesn't imply the existence of a threshold for effect.

58. The Committee is of the view that potency estimates can be of pragmatic use as an aid to prioritising carcinogenic substances (e.g. for risk re-evaluation) but considers that such potency estimates do not provide a quantitative estimate of risk. Although potency estimates can be used to rank chemicals within a particular group (such as structurally related groups of putative genotoxic chemicals), extrapolating

from high to low dose and from animals to humans introduces sources of uncertainty.

59. The Committee recognises that the TTC approach provides a pragmatic means of assessing whether exposure to a chemical is of low concern or whether further testing is required. However, the Committee reiterates that the TTC is not a replacement for data on any chemical under consideration but could be used where data are lacking or insufficient, to help in reaching a decision.

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